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Project Report: Bioextraction of Nutrients and Micronutrients from Minerals

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Project Progress

Fe, Mn, Zn, Ni, Cu, Co, and Mo are extremely low in abundance in natural waters, but each of these metals is used in bacterial enzymes, coenzymes, and cofactors. While it is well known that microbes secrete siderophores to extract Fe from their environment, it is not understood how these siderophores attack minerals to provide the FeIII, nor is it understood how bacteria extract other micronutrients. In previously reported work, we have shown that microbes can mobilize Mo (*Azotobacter vinelandii*), Ni (*Methanobacterium thermoautotrophicum*), and Cu (*Bacillus sp.*) from silicates. In addition, we have observed that macronutrients such as P can be extracted from earth materials and that elements found in minerals such as S and Fe can be used as electron acceptors. Our work investigates the mechanisms and isotopic effects of these processes as well. Specifics are given below.

We showed that methanogens can grow without dissolved Ni as long as Ni is present in a solid substrate. Over the last year we tested whether this growth was due to 1) release of a ligand that extracted Ni, 2) release of lysates after cell death, 3) release of extracellular proteins that interact with the Ni substrate, or 4) pH changes associated with growth of the methanogen. Graduate student L. Hausrath has clearly shown that the Ni extraction that occurs is due to pH changes related to utilization of carbon dioxide. We have found little to no other evidence for how methanogens condition their environment for growth. We have similarly observed no evidence for release of ligands by hyperthermophiles living in medium without dissolved *W.*

Anabaena, a cyanobacterium, was grown with fluorapatite as sole phosphorous (P−) source for 7 days. An inorganic control, apatite + medium was also run without inoculation. At the end of the experiment, fluorapatite grains from the various treatments were compared using scanning electron micrograph (SEM). Enhanced etching of the apatite in the presence of the cyanobacterium as compared to the abiotic control accompanies the enhanced clumping of the *Anabaena* when grown in the absence of soluble P. We are still investigating what controls the clumping and the etching.

We continue to analyze respiration of Fe oxides using an *in vitro* model for *Shewanella oneidensis*. A paper was submitted to *Environ. Sci. Technol.* on this topic. In addition, we have been investigating the isotopic fractionation of Cu during bacterial oxidation of chalcocite, and we are writing a paper on this topic.

Highlights

- Of the metal micronutrients, Fe, Mo, Ni, and W, we have found that the prokaryotes studied only release ligands to extract Fe and Mo: no ligands have been found to extract Ni and W by methanogens and hyperthermophiles, respectively. We hypothesize that these latter two types of organisms may live upon a chemical reaction that yields energy at a low enough level that it is not energetically feasible to release high affinity ligands.
- Aqueous copper in leach fluids released during abiotic oxidation of chalcocite ore are isotopically heavier (δ^{65}Cu = 5.3‰) than the initial chalcocite starting material (δ^{65}Cu = 2.6‰). This fractionation is attributed to the precipitation of secondary copper minerals. In the abiotic experiments, the heavier isotopic signatures of aqueous copper can be explained by mass balance between the starting material, aqueous copper, and the new precipitates. In contrast, the copper isotopic composition of aqueous copper in the similar experiments inoculated with Thiobacillus ferrooxidans is similar to the starting material throughout the leach cycle; however, the residual materials become significantly lighter for the last period of leaching (δ^{65}Cu = 0.6‰). This change in the isotopic signature of precipitated copper minerals can only be explained by incorporating a sink for isotopically heavy copper (δ^{65}Cu = 5.6‰) in the mass balance: this sink is inferred to be uptake into the bacteria cells. Mass balance is achieved for the biotic experiments when the bacteria, the precipitants, and the aqueous copper are considered. The generation of 'heavier' leach solutions during oxidation in these experiments would suggest that minerals precipitating in later stages of supergene enrichment processes (in naturally oxidative environments) would possess heavier copper isotopic signatures. The average δ^{65}Cu of five supergene samples analyzed are isotopically heavy and these results are in accord with other analyses of supergene minerals found in the literature.